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Lymphocyte enzyme activities in immunodeficiency syndromes with particular reference to common variable hypogammaglobulinaemia

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SUMMARY

Circulating non-T lymphocytes had higher activities of 5′nucleotidase (plasma membrane), neutral α-glucosidase (endoplasmic reticulum) and basal leucine amino-peptidase than did T lymphocytes. Activities of catalase (peroxisomes), malate dehydrogenase (mitochondria), lactate dehydrogenase (cytosol) and *N*-acetyl-β-glucosaminidase, β-glucuronidase and acid phosphatase (lysosomes), were similar in the lymphocyte subfractions. Lymphocyte 5′nucleotidase (plasma membrane) in patients with common variable hypogammaglobulinaemia is much lower than normal. However, the decrease is less marked in X-linked hypogammaglobulinaemia, chronic lymphatic leukaemia or protein loosing enteropathy or in lymphocytes isolated from cord blood. Cells from patients with nephrotic syndrome had normal levels of 5′nucleotidase. Other plasma membrane marker enzymes (γ-glutamyl transferase, leucine amino-peptidase) were normal in lymphocytes from patients with common variable hypogammaglobulinaemia. There is a selective reduction of mitochondrial (malate dehydrogenase) and cytosolic (lactate dehydrogenase) enzymes, with normal activities of lysosomal, peroxisomal and endoplasmic reticulum enzymes, in patients with common variable hypogammaglobulinaemia. The lymphocyte subcellular organelles in normal subjects and patients with common variable hypogammaglobulinaemia have similar properties on sucrose density gradient centrifugation. It is suggested that lymphocytes from patients with common variable hypogammaglobulinaemia show a specific enzymopathy and that this is not simply a reflection of cellular immaturity.

Keywords lymphocytes immunodeficiency syndromes 5′-nucleotidase lysosomes plasma membrane mitochondria

INTRODUCTION

Lymphocytes from patients with common variable hypogammaglobulinaemia (CVH) have low 5′nucleotidase activity (Johnson *et al.*, 1977; Webster *et al.*, 1978; Rowe *et al.*, 1980; Smith *et al.*, 1982). The decrease in 5′nucleotidase activity could be due to the presence of circulating immature lymphocytes, a defect in turnover of the enzyme (either increased breakdown or decreased synthesis) or to an abnormality in the structure of the enzyme protein. Other organelle enzyme activities, especially of the plasma membrane, were therefore studied in lymphocytes from patients

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with various immunodeficiency syndromes to determine if the plasma membrane enzyme alteration was specific. In addition these activities were assayed in lymphocyte subclasses from normal adults and in cord blood lymphocytes.

MATERIALS AND METHODS

Patients. Control subjects were healthy laboratory personnel. Patients with X-linked hypogammaglobulinaemia (XLH) were aged 21–26 years and were receiving regular injections of γ-globulin supplements (plasma IgG, 2·78 ± 0·67 g/l). Patients with common variable hypogammaglobulinaemia (CVH) were aged 25–57, mean 40 years, (plasma IgG, 1·76 ± 0·93 g/l). Both groups of hypogammaglobulinaemia patients had IgA and IgM levels of < 0·1 g/l. Patients with chronic lymphatic leukaemia (CLL) were not receiving chemotherapy at the time of study. All had B cell leukaemia and none showed evidence of a blast crisis.

Patients with protein losing enteropathy showed a marked elevation of faecal excretion of ^{51}Cr (4·7–12·9%; normal range < 1%) following i.v. administration of $^{51}CrCl_3$ (Waldmann, 1961). Two patients had intestinal lymphangiectasia and one patient had extensive small bowel Crohn's disease. All patients had reduced lymphocyte counts. Patients with nephrotic syndrome had 24 h protein losses ranging from 7·1 to 14 g with reduced serum IgG levels (2·7–5·1 g/l; normal range 6–16 g/l). Cord blood was collected from healthy infants immediately after delivery of the placenta. These studies were approved by the Harrow Health Authority Ethical Committee.

Isolation of mixed lymphocytes (Böyum, 1968). Blood (20–80 ml) was defibrinated and, after dilution with an equal volume of 0·15 M NaCl and 2 vols of RPMI medium 1640, pH 7·4 (GIBCO Biocult Ltd), 20 ml aliquots were layered onto 10 ml Lymphoprep (Uniscience Ltd., Cambridge). After centrifugation at 630*g* for 15 min at room temperature, the lymphocytes collected at the interface and were mixed with an equal volume of 0·2 M sucrose containing 1 mM Na_2 EDTA, pH 7·4 and 5 u/ml heparin (sucrose medium) and centrifuged at 1,800*g* for 10 min. Contaminating erythrocytes were lysed with 3 ml distilled water for 45 s, followed by the addition of an equal volume of 0·4 M sucrose medium. The lymphocytes were washed by resuspension and centrifugation (1,800*g* for 10 min) in 30 ml sucrose medium. The pellet was resuspended in 0·2 M sucrose medium and disrupted with 25 strokes of a tight fitting (type B) pestle in a Dounce homogenizer (Kontes Glass Co., Vineland, New Jersey, USA).

Isolation and T and non-T lymphocytes (Gmeglig-Meyling & Ballieux, 1977). A mixed lymphocyte suspension (2·5 ml), obtained as above, was mixed with 1·5 ml fetal calf serum (GIBCO Biocult Ltd) and 3 ml neuraminidase treated sheep erythrocytes (Tissue Culture Services) in 0·15 M NaCl added. The mixture was then layered onto a 5 ml Ficoll-Metrizoate (Uniscience) gradient and centrifuged at 630*g* for 35 min. The non-T cells, collected from the interface, were mixed with an equal volume of 0·2 M sucrose medium and recentrifuged (1,800*g* for 10 min). This pellet was resuspended in 3 ml sucrose medium and disrupted with a Dounce homogenizer. The T cells, with adherent sheep erythrocytes, were resuspended in 50 mM Tris-HCl buffer, pH 7·2 containing 1·42 mM NH_4Cl and maintained at 4°C for up to 20 min to lyse the erythrocytes. The T cells were then harvested as described above and homogenized in 0·2 M sucrose medium.

Analytical subcellular fractionation by zonal density gradient centrifugation. An aliquot of the lymphocyte homogenate was layered onto a 28 ml sucrose density gradient extending, linearly with respect to volume, from a density of 1·05 g/cm^3–1·28 g/cm^3, and resting on a 6 ml cushion of 1·32 g/cm^3 in a Beaufay automatic zonal rotor as described previously (Rustin & Peters, 1978). All gradient solutions contained 1 mM Na_2 EDTA, pH 7·4 and 5 u/ml heparin. The rotor was run at 35,000 r/min for 35 min and, after slowing to 8,000 r/min, some 16 fractions were collected in tared tubes, weighed and their densities determined (Peters, 1976).

Enzymic analysis. The lymphocyte homogenates and gradient fractions were assayed for the following enzymes (with organelle shown between parentheses): 5′nucleotidase (plasma membrane), lactate dehydrogenase (cytosol), particulate malate dehydrogenase (mitochondria), neutral α-glucosidase (endoplasmic reticulum), *N*-acetyl-β-glucosaminidase (lysosomes), catalase (peroxisomes). Details of the methods for these and other enzyme assays have been reported previously (Shah, Webster & Peters, 1983). β-Glucuronidase (Seymour & Peters, 1977) and neutral esterase

(Adebono, Coates & Cortner, 1978) were assayed with, respectively, 4-methylumbelliferyl β-D-glucuronide and 4-methylumbelliferyl butyrate derivatives, as substrates. All assays were validated for optimal pH, buffer and substrate concentrations and were linear with respect to incubation time and lymphocyte homogenate protein concentration. Samples were stored at −20°C for up to 2 months without significant loss of activity. Protein was assayed by a modification (Schacterle & Pollack, 1973) of the Lowry procedure with bovine serum albumin as standard. DNA was assayed fluorimetrically (Kapuschinski & Skoczylas, 1977) with calf thymus DNA (Type III Sigma Chemical Co) as standard.

Materials. γ-Glutamyl-7-amino-4-methyl-coumarin and L-leucine-7-amino-4-methyl coumarin were purchased from Uniscience Ltd., Cambridge, UK. 2[^{3}H]-Adenosine 5′monophosphate was from Amersham International, UK. Glycyl–glycine, Ammediol (2 amino-2 methyl 1,3, propandiol), piperazine, adenosine 5′monophosphate and 2 glycerophosphate were from Sigma Chemical Co. Ltd., Poole, Dorset, UK. All other reagents were of Analar grade from BDH Ltd (Poole, Dorset).

RESULTS

Enzyme activities in normal lymphocytes (Tables 1 & 2)

For six enzymes assayed, the activities in the mixed cell population were similar to those in the subclasses. However, the activities of 5′nucleotidase, malate dehydrogenase, *N*-acetyl-β-glucosaminidase and β-glucuronidase were considerably less in the lymphocyte subfractions than in the mixed cell population. There was greater acid phosphatase and catalase activity in the lymphocyte subclasses compared with the mixed cell population. There was also significant increase of the protein content of the lymphocyte subfractions. Most enzyme activities were similar in T and non-T lymphocytes, although the activity of 5′nucleotidase, neutral α-glucosidase and leucine amino

Table 1. Lymphocyte enzyme activities

Marker enzyme (organelles)	Mixed lymphocytes	T lymphocytes	Non-T lymphocytes
5′nucleotidase (plasma membrane)	112±30	25·7±5·6	44·9±5·9†
Catalase (peroxisomes)	371±90	895±275	999±207
Neutral α-glucosidase (endoplasmic reticulum)	3·88±0·57	3·82±0·36	5·86±0·62*
Lactate dehydrogenase (cytosol)	6,570±1,960	5,310±1,150	4,810±570
Malate dehydrogenase (mitochondria)	10,400±2,300	4,610±900	6,590±1,510
N-acetyl-β-glucosaminidase (lysosomes)	39·1±6·8	15·7±2·7	30·1±8·0
Acid phosphatase	39·4±5·7	115±41	112±23
β-glucuronidase	30·9±3·2	15·9±2·7	21·6±3·5
Leucine amino-peptidase	47·1±12·2	10·6±2·7	41·7±11·1*
γ-glutamyl transferase	13·1±2·8	22·9±2·8	18·3±5·5
Neutral esterase	24·0±9·3	37·1±15·3	43·2±9·1
Protein (mg/mg DNA)	381±107	151±61	92·2±12·4

Results show mean ±s.e. for four to six samples assayed in duplicate expressed as munits of activity/mg DNA. Mean recovery of lymphocyte DNA following erythrocyte rosetting, 84·7%. Statistical analysis between T and non-T lymphocytes by paired *t*-test: *$P<0.05$; †$P<0.02$.

Table 2. Comparison of lymphocyte enzyme activities in cells from normal adults and cord blood

Enzyme	Adult controls	Cord blood
5′nucleotidase	4·51±0·53 (14)	0·78±0·14† (5)
γ-glutamyl transferase	0·92±0·28 (15)	1·08±0·22 (5)
Leucine amino-peptidase	2·53±0·66 (14)	2·55±0·87 (5)
N-acetyl-β-glucosaminidase	1·72±0·18 (15)	1·18±0·24 (5)
Acid phosphatase	2·93±0·48 (15)	3·45±0·68 (5)
β-glucuronidase	2·20±0·12 (14)	0·55±0·07† (5)
Neutral α-glucosidase	0·39±0·07 (14)	0·30±0·02 (5)
Catalase	14·8±2·07 (12)	73·3±17·2† (5)
Malate dehydrogenase	648±88 (11)	470±96 (5)
Lactate dehydrogenase	313±35 (13)	382±41 (5)
Myeloperoxidase	0·06±0·01 (7)	0·13±0·03* (4)
Protein (mg/mg DNA)	20·9±3·2 (14)	13·6±5·4 (5)

Results show mean ±s.e. for (*n*) subjects; expressed as munits/mg protein; statistical analysis by Student's *t*-test: *$P<0.02$; †$P<0.001$.

peptidase activities were significantly greater in the non-T cells. The higher activity of neutral esterase in the T lymphocytes was not statistically significant.

The activities of 5′nucleotidase and β-glucuronidase were lower in cord blood cells than in adult lymphocytes but the activities of catalase and myeloperoxidase were greater. The activities of the other enzymes, in particular the dehydrogenases, were similar in the two groups.

Enzyme activities in lymphocytes from patients with immunodeficiency syndromes (Tables 3 & 4)
5′Nucleotidase activity is significantly lower in lymphocyte from patients with the immunodeficiency syndromes, especially CVH, but not in the patients with the nephrotic syndrome. In contrast, γ-glutamyl transferase and leucine amino-peptidase, also plasma membrane enzymes, showed no significant alteration in the immunodeficient patients. Basal leucine amino-peptidase activity which is also partly localized to the plasma membrane was also present in normal amounts. Low activity of myeloperoxidase, an enzyme not normally present in lymphocytes, provided evidence of a low level of contamination in all lymphocyte preparations in all patient groups. Non-specific esterase showed normal activities in lymphocytes isolated from CVH. The protein content of the cells expressed as mg/mg DNA also showed no significant differences between the various patient groups.

Of three lysosomal marker enzymes assayed (*N*-acetyl-β-glucosaminidase, β-glucuronidase and acid phosphatase), the activities of *N*-acetyl-β-glucosaminidase and acid phosphatase were similar in all patient groups. β-Glucuronidase activity was lower in the patients with chronic lymphatic leukaemia than controls but was normal in CVH. Neutral α-glucosidase (endoplasmic reticulum) activity was similar in all patient groups. The activity of catalase was higher in all patient groups, especially XLH. Activity of both dehydrogenases was lower in CVH patients but lactate dehydrogenase activity in the patients with nephrotic syndrome was increased.

Subcellular distribution of principal organelle marker enzymes in lymphocytes from control subjects and patients with CVH (Figs 1 & 2)
On sucrose density gradient centrifugation lactate dehydrogenase showed a predominantly soluble localization with very little particulate activity. The decrease in relative frequency reflects the marked reduction in activity in the CVH lymphocytes. 5′Nucleotidase shows a skewn peak with a modal density at 1·13 g/cm^3 with a striking reduction in the enzyme relative frequency in the CVH patients. Note that the modal density of the activity is similar in both control subjects and patients. Malate dehydrogenase shows a bimodal distribution with a small, but significant decrease in the

Table 3. Enzyme activities of control and patient lymphocytes

Enzyme	Adult controls	Primary immunodeficiency		Secondary immunodeficiency		
		XLH	CVH	CLL	Protein losing enteropathy	Nephrotic syndrome
5'nucleotidase	4·51 ± 0·53 (14)	1·73 ± 0·82* (5)	0·19 ± 0·05† (8)	0·51 ± 0·16† (5)	0·92 ± 0·76* (3)	1·98 ± 1·27 (4)
γ-glutamyl transferase	0·92 ± 0·29 (15)	0·57 ± 0·35 (5)	0·81 ± 0·26 (7)	0·35 ± 0·07 (5)	1·52 ± 0·11 (3)	0·94 ± 0·48 (4)
Leucine amino-peptidase	2·53 ± 0·66 (14)	5·01 ± 2·81 (3)	2·20 ± 0·97 (7)	1·58 ± 0·45 (4)	2·29 ± 1·42 (3)	2·88 ± 0·26 (4)
Myeloperoxidase	0·06 ± 0·01 (7)	0·09 ± 0·01 (3)	0·11 ± 0·03 (8)	0·04 ± 0·02 (4)	0·11 ± 0·03 (3)	0·04 ± 0·01 (3)
Neutral esterase	8·01 ± 1·44 (7)	ND	8·33 ± 1·30 (9)	ND	ND	ND
Protein (mg/mg DNA)	20·9 ± 3·2 (14)	28·7 ± 9·0 (4)	34·9 ± 9·6 (8)	30·2 ± 8·5 (5)	14·7 ± 2·4 (3)	11·3 ± 6·4 (4)

Results are expressed as mu/mg protein. The mean ± s.e. is shown with the number of patients studied in parentheses. ND = not determined. XLH = X-linked hypogammaglobulinaemia; CVH = common variable hypogammaglobulinaemia. Statistical significances from control values by Student's *t*-test: $*P < 0{\cdot}01$; $\dagger P < 0{\cdot}001$.

Table 4. Enzyme activities of control and patient lymphocytes

Enzyme	Adult controls	Primary immunodeficiency		Secondary immunodeficiency		
		XLH	CVH	CLL	Protein losing enteropathy	Nephrotic syndrome
N-acetyl-β-glucosaminidase	1·72±0·18 (15)	2·62±1·19 (3)	1·69±0·35 (7)	1·29±0·31 (5)	1·89±0·50 (3)	2·52±0·64 (4)
Acid phosphatase	2·93±0·48 (15)	4·28±1·33 (3)	3·94±0·81 (7)	3·63±0·66 (5)	5·65±3·18 (3)	6·57±2·29 (4)
β-glucuronidase	2·20±0·12 (14)	ND	1·80±0·28 (7)	1·12±0·12§ (5)	2·17±0·66 (3)	2·04±0·34 (4)
Neutral α-glucosidase	0·39±0·07 (14)	0·40±0·17 (3)	0·18±0·06 (7)	0·60±0·10 (5)	0·31±0·15 (3)	0·31±0·05 (4)
Catalase	14·8±2·1 (12)	83·2±13·7§ (3)	30·2±8·1* (7)	24·4±8·4 (5)	45·4±15·1‡ (3)	36·8±15·0† (4)
Malate dehydrogenase	648±88 (11)	793±290 (3)	373±97* (7)	581±152 (5)	435±162 (3)	729±72 (4)
Lactate dehydrogenase	313±35 (13)	270±156 (4)	97±12 (7)	211±35 (5)	246±81 (3)	516±82† (4)

Results are expressed as mu/mg protein. The mean±s.e. is shown with the number of patients studied in parentheses. ND=not determined. XLH & CVH; see footnote to Table 3. Statistical significances from control values by Student's *t*-test: $*P<0·05$; $†P<0·02$; $‡P<0·01$; $§P<0·001$.

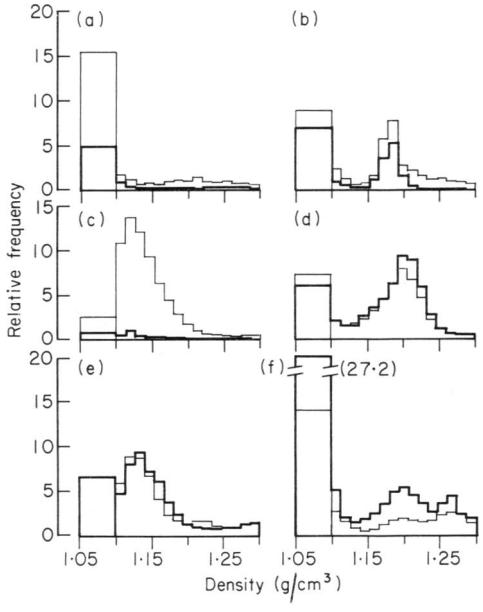

Fig. 1. Subcellular fractionation studies on lymphocyte homogenates isolated from blood of normal subjects and patients with CVH. Relative frequency–density distributions for six marker enzymes in control (thin line) and patient (thick lines) experiments. Relative frequency is calculated by multiplying frequency values for the patients lymphocyte fractions by the ratio of specific activity of patients homogenates to that of the control homogenate. (a) Lactate dehydrogenase; (b) malate dehydrogenase; (c) 5′-nucleotidase; (d) *N*-acetyl-β-glucosaminidase; (e) neutral α-glucosidase; (f) catalase.

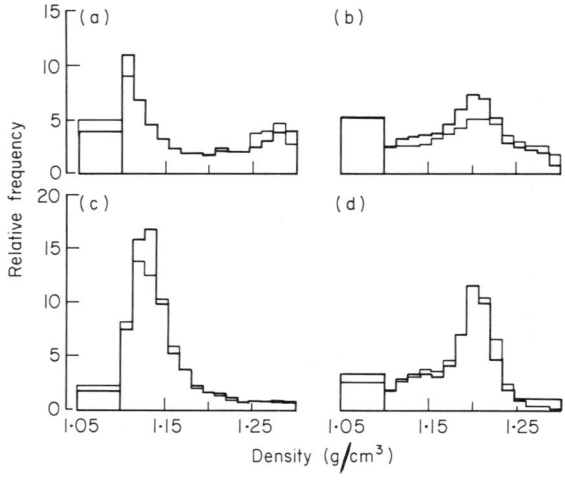

Fig. 2. Subcellular fractionation studies on lymphocytes isolated from blood of normal subjects and patients with CVH. (a) DNA; (b) acid phosphatase; (c) γ-glutamyl transferase; (d) basal leucine amino-peptidase. Further details as in Fig. 1.

relative frequency of this enzyme at both locations. *N*-Acetyl-β-glucosaminidase shows a broad peak with an equilibrium density of 1.20 g/cm^3 with some soluble activity in both patient groups. Catalase shows a marked increase in relative frequency in the CVH lymphocytes. All three components, particularly that at density 1·20 g/cm^3, show an increase in the patients. The distribution of neutral α-glucosidase is similar in distribution in controls and patients.

DNA is distributed mainly with the original sample layer, but some has sedimented into the

gradient but the distribution pattern were similar in the two patient groups. The distributions of acid phosphatase, γ-glutamyl transferase and basal leucine amino-peptidase were similar in patients and control subjects.

DISCUSSION

The results confirm that 5'nucleotidase activity is low in lymphocytes from patients with CVH (Johnson *et al.*, 1977; Edwards *et al.*, 1978) but this is less marked in XLH. Reduced enzyme activity in the patients with secondary immunodeficiency syndromes associated with CLL or due to excessive intestinal or urinary loss of protein might suggest that it is a secondary phenomena. However, the decrease in enzyme activity in these patients is much less than observed in patients with CVH. Another explanation of the reduced 5'nucleotidase levels is immaturity of the lymphocytes (Matamoros *et al.*, 1979). In favour of this hypothesis is that immature lymphocytes from cord blood have lower activities of 5'nucleotidase (Kramers *et al.*, 1977), confirmed in the present study. The reduced activity in the lymphocytes from patients with CLL is similar to that previously reported (Quagliata *et al.*, 1976) and is also consistent with cell immaturity. Patients with protein losing enteropathy, unlike those with nephrotic syndrome, have more immature lymphocytes and they have significantly reduced 5'nucleotidase activities. Although cell immaturity may be a partial explanation of the reduced 5'nucleotidase activity in patients with primary immunodeficiency syndrome, particularly the patients with XLH, the activity in CVH is much more reduced.

The reduced 5'nucleotidase activity seems to be selective as γ-glutamyl transferase, and leucine amino-peptidase, also plasma membrane enzymes, show normal levels. Adenosine diphosphatase, also localized to the plasma membrane, has normal activity in patients with primary immunodeficiency syndromes (Smith *et al.*, 1982). The subcellular fractionation studies confirm the location of these marker enzymes to the plasma membrane in the primary immunodeficiency patients and, in particular, shows that the equilibrium density of the membranes in the patients and controls is similar. This implies a similar membrane composition and supports the concept of a selective enzyme deficiency rather than a generalized lymphocyte plasma membrane abnormality.

The selectivity of the abnormalities in CVH is supported by the findings that the activity of three lysosomal enzymes is normal in the primary immunodeficiency syndromes. Reduced acid hydrolase activities, excepting β-glucuronidase, in lymphocyte from patients with CLL (Crockard, Lewis & Bridges, 1979) have not been confirmed in the present study. The normal activity of neutral α-glucosidase, an endoplasmic reticulum marker (Peters, 1976; Peters & Seymour, 1978), and its normal distribution in the sucrose density gradients suggests that this organelle is normal in CVH. Lymphocyte catalase activity was increased in the patients with both primary and secondary hypogammaglobulinaemia including CVH. Catalase is found in erythrocytes at high concentrations and the increased activities in the patients lymphocytes may represent, in part, increased erythrocyte contamination. This could also account for the apparent increase in lymphocyte catalase activity following isolation of T and non-T subfractions.

The two dehydrogenases, particularly lactate dehydrogenase, were selectively reduced in CVH. Lymphocyte lactate dehydrogenase isoenzyme studies (Matamoros, Abad & Webster, 1982) noted a relative deficiency of a LDH isoenzyme. Our results suggest that there is also an absolute deficiency of LDH in CVH. Both the soluble and mitochondrial isoenzymes of malate dehydrogenase were low in CVH and this would have a profound affect on lymphocyte glycolytic metabolism. These findings do not support the immaturity hypothesis.

Interpretation of enzymic studies on lymphocyte preparation from various patient groups may be confounded by differential isolation of T and non-T lymphocytes or by variable contamination with other leucocytes. However, the differences in the patient groups could not be explained by differing proportions of lymphocyte subtypes. The higher 5'nucleotidase activity in non-T than T cells, noted previously (Rowe *et al.*, 1979) could not account for the low enzyme activity in the patients lymphocytes even if only T cells were present. Note that the activities of the two dehydrogenases are similar in the T and non-T lymphocyte.

Previous reports have claimed that neutral α-glucosidase and neutral esterase are specific

markers for B (Philip *et al.*, 1982) and T (Mueller *et al.*, 1975; Totterman, Rank & Hayry, 1977) lymphocytes, respectively. Our data suggest that the distinction is not complete. The activity of these enzymes is also normal in CVH, indicating that there has not been a gross alteration in the lymphocyte populations in these patients.

Although there was some lymphocyte contamination by polymorphonuclear leucocytes and monocytes it varied in the different patient groups. Comparison of the specific activity of myeloperoxidase in pure populations of neutrophils (Rustin & Peters, 1979) indicates a 10% contamination of control cells and, no more than a 20% contamination of CVH, by neutrophils. Neutrophilic leucocytes contain significantly less (2%) 5'nucleotidase than lymphocytes (Rustin & Peters, 1979) and this increased contamination could not account for the reduced 5'nucleotidase activity in the lymphocytes isolated from immunodeficient patients.

These results emphasize the specificity and selectivity of alterations in 5'nucleotidase in patients with CVH. The organelle pathology of these lymphocytes are not grossly abnormal but it might be anticipated that they would show abnormalities in energy metabolism.

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